THE EFFECTS OF CONDITIONS OF HOMOGENIZATION ON THE CONTENTS OF NUCLEI ISOLATED FROM RAT LIVER

An abstract of a Thesis by
Louis T. Biehle
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Drake University

The problem. To determine the effects of degree of homogenization, on DNA, RNA, and protein content of nuclei isolated from rat liver.

The procedure. Rat liver cells were broken with a Dounce homogenizer using variable numbers of strokes from 05 to 40. Nuclei were isolated, counted, measured and analyzed for nucleic acid and protein contents.

Finding. It was found that the amount of homogenization had a very little effect on the contents of the isolated nuclei.

Conclusion. Further homogenization has no effect on protein content of isolated nuclei. All of the protein must be leached out of the nuclei immediately. Nucleic acid content does not decrease as nuclear counts decrease thus fragmented nuclei are sedimented during the isolation process.

Recommendations. The conditions of rehomogenization could be varied to determine the effects on protein and nucleic acid content. It is also recommended the forces of sedimentation be varied to further study the fragmented of invisible nuclei.

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Louis T. Biehle

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by

Louis T. Biehle

Approved by Committee:

Harold D. Swanson
Chairman

Robert M. Kodama

Joe D. Woods

Earle L. Canfield
Dean of the School of Graduate Studies

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INTRODUCTION AND REVIEW OF LITERATURE

The importance of the methods of homogenization and centrifugation for the success of nuclear isolation has been well established. It is necessary to know what happens to the nuclei during the homogenization and centrifugation procedures. This thesis will measure the effects of different amounts of homogenization on the contents of isolated nuclei.

Liver tissue consists of several types of cells. Parenchymal cells, sinusoid endothelial cells, connective tissue cells (called stroma), bile duct cells and the Kupffer phagocytic cells have been described (Ham and Leeson, 1961). The literature suggests that the liver is about 60% parenchymal cells by volume (Daoust, 1958). Parenchymal cells have been found to contain nuclei that may have double or quadruple the volume of the basic nucleus; some parenchymal cells may be multinucleated (Greep, 1966). Many workers, starting with Beams and King (1942), have found that these variable nuclei are polyploid, have multiples of the usual chromosome number (Greep, 1966). As observed in fixed and stained sections, nuclei from various cell types differ in appearance. Parenchymal cell nuclei are ovoid to spherical (Ham, 1965); Kupffer cell nuclei are ovoid; nuclei from the endothelial cells display flattened dense nuclei. But the latter two types of nuclei are often indistinguishable (Greep, 1966).

The parenchymal nuclei were divided into classes by Beams and King (1942) and by Daoust and Contero (1959) on the basis of nuclear diameters as measured by ocular micrometry of stained and fixed sections of rat liver tissue. Three classes were described by Alfert (1958), corresponding to diploid, tetraploid, and octoploid cells with 1, 2, and 4 times the normal number of deoxyribonucleic acid.

Detailed classification of isolated nuclei was described by Antoine (1971), who distinguished 6 classes of liver nuclei based on diameters. The larger nuclei (classes 1-4) were assumed to be parenchymal cells. These larger nuclei were in greater abundance than smaller ones as would be expected from the literature (Daoust, 1958; Alfert, 1958; Falzone et al., 1962; and Albrecht, 1968). Classes 5 and 6 were assumed to be nonparenchymal (Antoine, 1971).

Falzone et al. (1962) found that the largest percentage of parenchymal nuclei in an adult rat are diploid and tetraploid, but that the octoploid and even those of higher ploidy were found in very small numbers.

With so many different kinds of nuclei in rat liver tissue it is possible that some of the methods of homogenization and centrifugation have not produced the best yield and purity of isolated nuclei. Chauveau, Moule, and Rouiller (1956) have suggested that the following criteria are important in the isolation of cell components: (A) homogeniety of the cell fraction, (B) preservation of its morphology and

biochemical composition, (C) sufficiently high yield.

Anderson (1955) has suggested that a number of factors may be expected to have a pronounced influence on the kinds and conditions of isolated nuclei. Much work has been done with varying the isolating media in the preparation of homogenates (Wilbur and Anderson, 1957; Schneider, 1948). Most investigators have used saline or sucrose media to prepare homogenates (Wilbur and Anderson, 1957). Schneider (1948) found that sucrose solutions are most efficient in separating nuclei from the other cellular organelles such as mitochondria than the saline solution alone. A sucrose medium alone causes distortion and contamination of the preparation, so Schneider and Peterman (1950) added calcium chloride and magnesium chloride to "harden" the nuclei without causing agglutination of the cytoplasm.

Early investigators used differential centrifugation for nuclear isolation (Wilbur and Anderson, 1951), Chauveau et al. (1956), point out that differential centrifugation, which is based on sedimentation rates, allow contamination because all of the particles migrate the same way. In comparison, density gradient centrifugation enables a complete separation to be made because only particles having a density higher than that of the medium sediment out during centrifugation; the other particles having a lower density migrate in the opposite direction. They also found the yield was much better and the nuclei were free from cytoplasmic contamination

and were morphologically well preserved. This was supported by Gill (1965).

The Chauveau method can result in some degree of cytoplasmic contamination, since those particles with a density equal to or higher than that of nuclei (free ribosomes) and which are initially near the bottom of the tube will sediment into the pellet with nuclei (Blobel and Potter, 1966). In the study of the Chauveau process by Maggio, Siekevitz and Palade (1963) a slight but definite contamination was shown. This contamination could be eliminated by layering a homogenate in 0.88 M sucrose medium over 2.2 M sucrose. The yield was reduced because of an accumulation of endoplasmic reticulum and mitochondria at the interface, which then tapped many nuclei (Blobel and Potter, 1966). Blobel and Potter (1966) found that by raising the sucrose concentration of the homogenate to a density sufficient to float the endoplasmic reticulum and mitochondria the interface problem was eliminated and the yield was improved, sometimes approached 90%.

Blobel and Potter (1966) found that the DNA of the nuclear pellet represented about 91% of the DNA of the filtered homogenate, but only 4.3% of the total RNA in the homogenate was recovered with the nuclei. If one assumes that no nuclear RNA was extracted during isolation and corrects for 100% recovery of nuclei, then the nuclear RNA would amount to only 4.7% of the total cellular RNA.

Disruption of cells in preparing homogenates has been accomplished in many ways; the most popular methods use the Dounce hand plunger or the Potter-Elvehjem motor driven teflon pestle. These plungers work on the principle of fluid turbulence and shearing which have been reported to bring about good tissue disruption (Anderson, 1956).

Antoine (1971) found that difference in number of strokes using a Dounce homogenizer had a profound effect on the yield and purity of isolated nuclei. The increasing number of strokes produced a decrease in concentration of almost all nuclear types, but a faster decrease of larger parenchymal nuclei.

Nuclei freed from disrupted cells are quite porous, allowing the entry or exit of even very large molecules (Anderson, 1956). Exposure of a nucleus to repeated conditions of turbulence and fluid movement might be expected to extract increasing amounts of any partially soluble or incompletely stabilized components. The experiments for this thesis were designed to analyze the effect of various degrees of homogenization on the contents of isolated rat liver nuclei.

METHODS AND MATERIALS

Adult male albino rats over 4 months of age of the Wistar strain were etherized and immediately decapitated. The livers were removed, washed in tap water and minced with scissors. Each minced liver was placed in 50 ml of 0.25 M sucrose in TKM (a buffered solution containing 0.05 M Tris-HCL, pH 7.5 at 20° C; 0.025 M KCL; 0.005 M $MgCl_2$) (Blobel and Potter, 1966). The procedures followed were those of Blobel and Potter (1966), except for type of homogenizer and the use of an angle instead of a swing bucket centrifuge head.

A Dounce homogenizer tube with a loose fitting Teflon plunger made of Kontes glass and having a clearance of 0.0123 mm at 0° C was used to prepare five homogenate preparations. The nuclear isolation was repeated for 5 separate experiments. All experiments with exceptions of nuclear counts and nuclear analysis were carried out at 0° to 4° C.

Each of the 5 experiments was carried out in the same way. The liver was homogenized for 5 strokes, the homogenate was strained through nylon mesh to remove connective tissue. Eight different batches of nuclei were isolated, with variations in the stroke number: a 5 ml sample of homogenate was extracted from the homogenizer after every 5 strokes of the plunger and then was prepared for nuclear isolation. As a result nuclear isolations were prepared at

5, 10, 15, 20, 25, 30, 35, and 40 strokes, representing 40 homogenate preparations for the 5 experiments.

The nuclear isolations were carried out by placing the 5 ml samples into centrifuge tubes and thoroughly mixing with a 10 ml sample of 2.3 M sucrose in TKM. From this mixture a 5 ml sample of homogenate was drawn off and placed in another centrifuge tube. All samples were kept in an ice bath at 0° C. The sample was then underlaid with 35 ml of 2.3 M sucrose in TKM by means of a syringe and a large needle; the tip of the needle was placed at the bottom of the tube slowly forcing the lighter homogenate upward. The tubes were then centrifuged in the Beckman L 3-40 Ultracentrifuge for 30 minutes at 40,000 rev/min in a Spinco 50.1 rotor (123,000 g av) at 0° to 4° C. After centrifugation, the tubes were removed and placed in an ice bath. From the centrifuge tube, the supernatant fluid was aspirated off and the materials adhering to the walls were removed by a spatula and tissue. The nuclear pellet was taken up in 10 ml of 0.25 M sucrose in TKM.

From the 10 ml of suspended pellet 3 ml of solution was drawn off and placed into test tubes for various microscopic examinations. Nuclear concentrations were determined with a hemocytometer (Falzone et al., 1962; Antoine, 1971). Two counts were taken and the average of the counts was used. To 1 ml of the nuclear sample 1 drop of 1% methyl green was added to facilitate observation of the nuclei while counts

were being made. Sizes of unstained nuclei were determined by an ocular micrometer on a Nikon phase contrast microscope with oil immersion. Diameters of 75 randomly chosen nuclei were measured from each of the samples and were placed into classes based on their size (Antoine, 1971). The following classes were used: class 1 involved all nuclei which ranged from 14.96 microns to 11.56 microns, class 2 involved all nuclei from 11.22 to 9.52 microns, class 3 involved all nuclei from 9.18 to 7.82 microns, class 4 was from 7.48 to 6.46 microns, class 5 was from 6.12 to 4.08 microns and class 6 was 4 microns and under (Antoine, 1971). To the remaining 7 ml of each sample was added an equal volume of cold 14% TCA (trichloroacetic acid) to precipitate the nuclear proteins and nucleic acids. The solution was sedimented with a desk centrifuge. From the test tube, the supernatant fluid was aspirated off and the pellet was washed in cold TCA twice. The suspension was then sedimented in a desk centrifuge and the liquid was removed. The pellet was suspended in 10 ml of 0.1 M NaOH and analysis of the contents determined colorimetrically.

Standard curves were prepared using duck erythrocyte DNA, purified RNA, and bovine albumin for protein. The nucleic acids and the protein were determined through the use of color reactions involving the pentose components of the nucleic acids and the amino acids of the protein. The standard curves were established on known concentrations of

the nucleic acids and protein by analysis of their absorbances. Since the curves were linear and the standard of .2 mg per ml which was used was variable from run to run, a value for the nuclear contents was obtained by the use of the formula:

$$(U/S)\ (Cs) = Cu$$

In this formula the U is the absorbance of the unknown, S is the absorbance of the standard, Cs is the concentration of the standard and Cu is the concentration of the unknown.

The contents of the sample were determined by the use of the Dische (1930) reaction for DNA, the Orcinol reaction for RNA (Glick, 1954) and the Folin-Ciocalteau reaction for protein synthesis (Lowry, et al., 1951; Bailey, 1967). All readings of absorbances were performed on a Coleman Junior Spectrophotometer. The DNA was read at 590 nm (Dische, 1930), RNA was read at 650 nm (Glick, 1954) and the protein was read at 650 nm (Lowry et al., 1951; Bailey, 1967).

Since the orcinol reagent for RNA also produces some color with DNA, the correction formula was used:

$$R = \frac{A - D\ K_2}{K_1}$$

In this formula R is the corrected concentration of RNA, D stands for the concentration of DNA as determined by the Dische reaction. A is the absorbance from the Orcinol

reaction on the unknown sample, K_1 is the ratio of absorbance to concentration as determined by the Orcinol reagent on the RNA standard, whereas K_2 is the ratio of absorbance to concentration as determined by the orcinol reagent with the DNA standard.

From the concentrations, DNA, RNA, and protein yields, and RNA/DNA, and protein/DNA ratios were determined.

The analysis of the data collected from the 5 homogenate preparations was based on the number of strokes with the plunger as compared to nuclear contents. Statistically this was done with use of linear regression analysis. All calculations for the linear regression were performed on a Hewlett-Packard calculator Model 9100-B, program number 09100-70803 as prepared by Prentice Hall, 1962. Correlation coefficients, also determined by the same computor program, were tested for significance as prescribed by Walpole and Meyers (1972). Confidence intervals for the slopes of regression lines were determined by the following formula, rearranged in Walpole and Meyers.

$$m \pm \quad t_{(.05,\ n-2)}\ (s_y)\ (s_x)^{-1}\ (1-r^2)^{\frac{1}{2}}\ (n-2)^{-\frac{1}{2}}$$

In this formula, m is the calculated slope of the regression line, t is the value from standard tables for 95% confidence level, and n-2 degrees of freedom, s_y and s_x are the standard deviations for the x and y values for the sample, r is the calculated correlation coefficient, and n is the number of

pairs of observations.

DATA AND DISCUSSION

Table 1 shows the results of the analysis of the DNA concentration by varying the degree of homogenization. From the overall regression of Table 4, one observes a negative slope of the line. This indicates that there is some slight decrease in DNA concentration as homogenization increases. This decrease in DNA concentration does not occur nearly as rapidly as the number of nuclei decreases. From Table 4 it was also found that the correlation between concentration and stroke number was very low. Antoine (1971) found a very high correlation between increasing homogenization and a corresponding decrease in nuclear types using nuclear measurements. He used 100 randomly chosen nuclei from each sample of homogenate. In the present work, only 75 randomly chosen nuclei were used. Since Antoine used higher nuclear counts his results would be more accurate on nuclear types and counts. The included data found in Table 12, however, substantiates Antoine's results. From this work one would expect to find that as the nuclear counts decreased that there would be a corresponding decrease in DNA concentration. This does not seem to be the case.

To test the hypothesis that DNA content of the isolated nuclear fraction does not change at the same rate with

increased homogenization as does number of nuclei, confidence limits for the slope of each regression were calculated. Data from Tables 1 and 12 were used for this calculation, omitting data from the one rat not represented in both tables. For each set of data, the values were expressed as fractions of the amounts isolated with only 5 strokes of homogenization. This allowed inclusion in one calculation of data from several experiments in which the absolute yield differed. It also made the slopes calculated for changes of numbers of nuclei and amounts of DNA comparable to each other. The regression line calculated for nuclear numbers had a slope of -0.0124, a Y intercept of 1.066, and a correlation coefficient of -.9105. This meant that a little over one percent of the nuclei were lost with every additional stroke of homogenization. The regression equation for amount of DNA isolated had a slope of -.0033, a Y intercept of -.0588 and a correlation coefficient of -.1734. Since the critical value for r, with 30 degrees of freedom, and at the 95% confidence level, is .362, the correlation of DNA contents with degree of homogenization is clearly not significantly different from zero. The calculated estimate of the slope, -.0033, would indicate that about a third of one percent of the DNA was lost with each additional stroke of homogenization.

The calculated confidence interval for the slope of nuclear numbers is from -.0103 to -.0146. The similar confidence limit for DNA contents is from -.0102 to +.0036.

Since the confidence interval for the slope of DNA change overlaps zero, this is another way of saying the same thing as indicated by the correlation coefficient; there is no ground for confidence, that the DNA content actually does decrease with homogenization. But despite the variability of the DNA measurements, the confidence limits for the slope of DNA change does not overlap the confidence interval for the slope of change in nuclear numbers. Whether or not the DNA content actually does decrease, it decreases significantly less rapidly than nuclear numbers.

The original expectation is not upheld. DNA content of the isolated nuclear fraction does not decrease as rapidly as do numbers of identifiable nuclei.

Blobel and Potter (1966) obtained an average yield of 91%. Their yield had a range from 76.6% to 97.4%. In Table 2 the percentage yields of DNA versus stroke number are listed. Most of the yields were acceptable and within the range of those obtained by Blobel and Potter (1966), except for rat 4 which gave a very low yield. The reasons for this low yield are not apparent. Rat 2 and 3 gave the best overall yield. Antoine (1971) found that the best yield and purity of parenchymal nuclei would be from 8 to 15 strokes with 10 strokes being optimum. Increasing the number of strokes also affected the purity and yield of the nuclei being isolated.

Since the slope of the nuclear loss does not correspond

Table 1. DNA contents in nuclei isolated by varying degrees of homogenization. The rows represent individual experiments; columns represent conditions of homogenization. Contents are expressed in mg of DNA per ml of sample.

RAT	05	10	15	20	25	30	35	90
1.	0.044	0.030	0.034	0.019	0.039	0.024	0.038	0.028
2.	0.074	0.059	0.058	0.072	0.061	0.061	0.073	0.077
3.	0.034	0.047	0.033	0.039	0.046	0.044	0.037	0.039
4.	0.103	0.086	0.057	0.085	0.037	0.040	0.066	0.052
5.	0.048	0.045	0.053	0.050	0.055	0.043	0.055	0.044

Table 2. Percent yield DNA in nuclei isolated by varying degrees of homogenization. No yield taken on RAT 1. Rows represent individual experiments; columns represent conditions of homogenization. Yield is expressed as a ratio of DNA contents of pellet to homogenate.

RAT	05	10	15	20	25	30	35	40
2.	0.944	0.744	0.732	0.909	0.770	0.770	0.921	0.972
3.	0.768	0.963	0.745	0.881	0.914	0.973	0.746	0.786
4.	0.877	0.732	0.702	0.775	0.576	0.672	0.678	0.538
5.	0.753	0.704	0.833	0.780	0.853	0.674	0.853	0.683

to the slope of the DNA loss and the results of the yield are very close to those obtained by Blobel and Potter (1966), then one would be concerned about the possible reasons behind the differences. It is possible that fragmented nuclei less than 0.2 microns in Diameter are being sedimented. The time and force present in the ultracentrifuge are great. Due to the viscosity of the solution that the particles which are sedimenting had to pass through; it is possible to determine the approximate sedimentation coefficient (S*) and to establish the particles found in the pellet. Bishop (1966) and Cline and Pyel (1971) stated the formula for figuring the S* value.

$$S^* = \frac{D_p - D_w}{(V_w)\ (w^2 t)} \cdot \frac{V_m\ (dM)}{(D_p - D_m)\ (R)}$$

In this formula D_p is the density of the particle, D_w is the density of water at 20^o C., V_w is the viscosity of water, V_m is the viscosity of the medium, dM is the distance that the particles move, D_m is the density of the medium, R is the average radius, and w^2t is the angular velocity of the centrifuge tube. Most of these values can be obtained from table of constants. The approximate S* value was obtained for minimal and maximal areas of the centrifuge tube that particles traveling needed to reach the wall of the tube. The minimal S* value as calculated was 2×10^5 with the maximal value being 5.5×10^5. Anderson (1966) illustrated a

diagramatic presentation of distribution of subcellular components as a function of sedimentation rate and banding density. The centrifugal systems now available are capable of making separations on the basis of either particle sedimentation rate or banding density. If a particle has a sedimentation coefficient and a banding density not shared by subcellular particles, these particles can be isolated in a pure state. Anderson's chart indicated that nuclei have an S* value of approximately 10^7, while particles of mitochondrial size have a maximum S* value of 10^5.

Assuming that the densities are the same then any nuclear fragments larger than mitochondria should have sedimented with the nuclei, but then any nuclear ribosomes, or particles of that or smaller size, would not have come down with the pellet, but would have remained at the interface. From this chart it was ascertained that nuclei and large nuclear fragments would sediment but the other subcellular components would not. Anderson's values were derived from a variety of experiments by different methods, but they represent aqueously isolated components. The gap in S values is so great that even if his values are off by a factor of 10 the same conclusions will hold true. The fragmented nuclei (there would be a large number at maximum stroke number) must not have had much protein or RNA extracted from them, since their concentrations varied only slightly. Thus these components are not held in by the nuclear membrane

because of the porosity of the membrane and turbulence of the fluids, but by some kind of attachment to the nuclear solid phase.

Table 3 contains the data comparing the concentrations of proteins to varying degrees of homogenization. Very little published data could be obtained for comparison in this area. Finical (1971) found a high Protein/DNA ratio than Wilbur and Anderson (1951). Finical stated that one of the advantages of the nonaqueous method of isolation is that the water soluble proteins are not lost. Finical found a 7.9 ratio and Wilbur and Anderson found a 5.1 ratio. Table 9 illustrates the protein/DNA ratio obtained by varying homogenization. These values are comparable to those obtained by Wilbur and Anderson. From the difference in the two ratios there must be some protein leaching during the isolation process. The protein loss that was obtained in the experiments was an average of 0.44 mg/ml with a range from 0.21 to 0.70 mg/ml. The loss for each individual experiment was very close. It was found that the loss of protein occurred during the first homogenization. Therefore all of the water soluble proteins are removed early in the homogenization and further homogenization is not important in determining protein content of nuclei.

Table 4 shows the regression analysis of the first 3 tables. In column a is the regression of Table 1, column b is the regression of Table 2, and column c is the regression

Table 3. Concentration of protein in nuclei isolated by various degrees of homogenization. Rows represent individual experiments and columns represent conditions of homogenization. The contents are expressed in mg per ml.

RAT	05	10	15	20	25	30	35	40
1.	0.140	0.170	0.156	0.118	0.140	0.200	0.067	0.067
2.	0.055	0.121	0.133	0.111	0.108	0.072	0.087	0.106
3.	0.136	0.112	0.191	0.144	0.146	0.140	0.140	0.128
4.	0.223	0.146	0.145	0.098	0.112	0.133	0.154	0.125
5.	0.163	0.169	0.129	0.148	0.183	0.131	0.162	0.178

Table 4. Regression analysis of variations in nuclear contents with variation in number of strokes of homogenization. Symbols are y = mx + b; where y is content in mg per ml; m is the slope of the line; x is the number of strokes from 05 to 40; b is the Y intercept; r represents the correlation coefficient. By t-test with samples of 5 pairs, a correlation coefficient of over .71 indicates the slope is significantly different from zero. Rows represent individual experiments and columns represent regression values.

Contents	Conc. DNA			Yield DNA (no values rat 1)			Conc. Protein		
RAT	r	b	m	r	b	m	r	b	m
1.	-.2185	.0361	-.0002	-----	-----	-----	-.3220	.1666	-.0013
2.	.0430	.0649	.00003	.0435	.8182	.0004	-.0747	.1020	-.0002
3.	.2160	.0377	.0001	.0943	.8380	.0009	.0753	.1409	.0002
4.	-.6924	.0996	-.0016	-.6912	.8360	-.0060	-.5133	.1824	-.0019
5.	.2827	.0475	.0001	.2825	.7406	.0019	-.0954	.1584	-.0002
mean regression	-.1178	-.00019	.0554	-.0937	.8112	-.0009	-.6587	-.0005	.1416

of Table 3. Symbols used are $Y = MX + b$, where Y is the concentration of the nuclear component; M is the slope of the line; x represents the variation in homogenization; b is the X intercept; and r is the correlation coefficient. Using the student t-test with samples of 5 pairs of correlation coefficient over 0.71 indicates that the slope is significantly different from 0. In observation of the data contained in this chart it can be seen that the data is not above the significance level. Therefore little correlation can be established. Antoine (1971) found a very high correlation with respect to numbers of nuclei and varying in the amount of homogenization that takes place. Since little correlation can be found in the experiment one must assume that there is little correlation between degrees of homogenization and nuclear contents. An explanation to what is happening is that the nuclei sedimenting carry everything with them, including the fragmented nuclei and nuclear contents.

Tables 5 through 12 in the appendix show the RNA concentration, RNA yields, protein yields, protein/DNA ratio, RNA/DNA ratio and the regression of the same. Here again the correlation was low but the results were comparable to those obtained by Blobel and Potter (1966) and Finical (1971).

CONCLUSION

From the work of Antoine (1971) it was found that nuclei could be divided into 6 classes on basis of their diameters; and the effects of conditions of homogenization can clearly be seen with increasing number of strokes which produce a decrease in concentration of all nuclear types. This work was substantiated.

During homogenization nuclei are being disrupted. While disruption is taking place very little protein is lost due to further homogenization. DNA is decreasing during the homogenization process, but not decreasing nearly as rapidly as the numbers of visible nuclei decreases. From the calculation of the S* values it was found that only nuclei or large nuclear fragments contain protein and nucleic acid. Therefore further homogenization has little effect on the contents of isolated nuclei.

The work on this thesis may be further extended by studying the effects of rehomogenization on types of nuclei and their contents. It is also recommended that a more thorough study be made of the invisible nuclear fragments at higher degrees of homogenization and the isolation process in the centrifuge be done at varying speeds.

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APPENDIX

Table 5. RNA concentration in nuclei isolated by various degrees of homogenization. Rows represent individual experiments and columns represent varying conditions of homogenization. Contents are expressed in mg of RNA per ml solution.

RAT	05	10	15	20	25	30	35	40
1.	.0029	.0002	.0006	.0042	.0029	.0010	.0008	.0002
2.	.0140	.0077	.0043	.0009	.0269	.0116	.0052	.0012
3.	.0045	.0018	.0014	.0025	.0045	.0004	.0112	.0003
4.	.0147	.0471	.0063	.0073	.0073	.0046	.0032	.0036
5.	.0115	.0104	.0084	.0115	.0134	.0079	.0492	.0189

Table 6. Yield of RNA in nuclei isolated by various degrees of homogenization. Rows represent individual experiments and columns represent varying degrees of homogenization. Yield is expressed as a ratio of the nuclear contents of the pellet as to the homogenate.

RAT	05	10	15	20	25	30	35	40
1.	-----	-----	-----	-----	-----	-----	-----	-----
2.	.0217	.0120	.0067	.0014	.0418	.0034	.0081	.0019
3.	.0277	.0111	.0032	.0154	.0277	.0055	.0690	.0018
4.	.0196	.0629	.0084	.0098	.0098	.0061	.0043	.0048
5.	.0451	.0408	.0329	.0450	.0525	.0526	.0310	.0740

Table 7. Protein yield in nuclei isolated by various degrees of homogenization. Rows represent individual experiments and columns represent varying degrees of homogenization. Yield is expressed as a ratio of the nuclear contents of the pellet as compared to the homogenate.

RAT	05	10	15	20	25	30	35	40
1.	-----	-----	-----	-----	-----	-----	-----	-----
2.	-----	-----	-----	-----	-----	-----	-----	-----
3.	.3091	.2545	.4341	.3273	.3318	.3182	.3182	.2909
4.	.2802	.1830	.1822	.1231	.1410	.1667	.1933	.1569
5.	.2998	.3106	.2369	.2715	.3357	.2411	.2981	.3267

Table 8. Regression analysis of variations in nuclear contents with variation in number of strokes of homogenization. Symbols are $y = mx + b$; where y is nuclear contents in mg per ml; m is the slope; x is the number of strokes from 05 to 40; b is the y intercept; r represents the correlation coefficient. Rows represent individual experiments and columns regression values.

Contents	Conc. RNA			Yield RNA			Yield Protein		
RAT	r	b	m	r	b	m	r	b	m
1.	.5479	.00009	.00014	-----	-----	-----	-----	-----	-----
2.	.0357	.0095	.00003	-.1272	.0169	-.0002	-----	-----	-----
3.	.4345	.0008	.0002	.4689	.0032	.0010	.0755	.00037	.3201
4.	-.5897	.0299	-.0009	-.5898	.0399	-.0011	-.5135	.2291	-.0024
5.	.5919	-.0001	.0008	.0049	.0428	.000004	-.0955	.2913	-.0003
mean regression	-.0267	.0092	-.00003	-.2643	.2719	-.0045	-.1367	.2821	-.00095

Table 9. Protein/DNA ratio from nuclei isolated by various degrees of homogenization. Rows represent individual experiments and columns represent varying degrees of homogenization.

RAT	05	10	15	20	25	30	35	40
1.	3.182	5.667	4.588	6.211	3.590	8.333	1.763	2.393
2.	.7432	2.051	2.293	1.542	1.771	1.180	1.192	1.377
3.	4.000	2.383	5.788	3.692	3.174	3.182	3.784	3.282
4.	2.165	1.694	2.544	1.154	3.057	3.301	2.335	2.388
5.	3.382	3.745	2.412	2.952	3.336	3.035	2.963	4.055

Table 10. RNA/DNA ratio as obtained from nuclei isolated by various degrees of homogenization. Rows represent individual experiments and columns represent varying degrees of homogenization.

RAT	05	10	15	20	25	30	35	40
1.	.0659	.0067	.0176	.2211	.0744	.0477	.0211	.0071
2.	.1892	.1305	.0741	.0125	.4410	.1902	.0712	.0156
3.	.1324	.1342	.0424	.0641	.0978	.0091	.3027	.0077
4.	.1427	.8263	.1105	.0860	.1989	.1144	.0486	.0688
5.	.2386	.2306	.1573	.0230	.2450	.1829	.8995	.4315

Table 11. Regression analysis of variations in nuclear contents with variation in number of strokes of homogenization. Symbols are $y = mx + b$; y is nuclear contents in mg per ml; m is the slope of the line; x is the number of strokes from 05 to 40; b is the y intercept; r is the correlation coefficient. Rows are experiments and columns are conditions of homogenization.

Contents	RNA/DNA ratio			Protein/DNA ratio		
RAT	r	b	m	r	b	m
1.	-.0080	.0652	-.00005	.0028	4.751	.0006
2.	.0726	-.1395	.00095	-.1305	1.670	-.0066
3.	.2543	.0667	.0027	-.1213	3.953	-.0119
4.	-.4586	.4493	-.0016	.4389	1.716	.0303
5.	.5389	.00027	.0141	-.3203	3.368	-.0125
mean regression	-.0771	.1833	-.0013	.0796	3.251	-.0107

Table 12. Nuclear concentrations isolated by varying degrees of homogenization. Rows represent individual experiments and columns represent varying conditions of homogenization. Total concentration equal 10^7 nuclei per MM^3.

RAT	05	10	15	20	25	30	35	40
1.	3.070	3.000	2.850	2.300	2.250	2.000	1.750	.1500
2.	3.500	3.450	3.380	3.430	3.200	2.430	2.500	2.180
3.	-----	-----	-----	-----	-----	-----	-----	-----
4.	5.075	4.625	3.950	4.200	3.650	3.625	3.425	2.975
5.	6.125	5.750	5.350	4.175	3.925	3.875	3.950	3.450